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(57) Abstract

In previous studies, Copolymer 1 given at a dose of 20 mg daily has been shown in a controlled study to decrease the relapse rate in multiple sclerosis (MS) by about 30 % over placebo. In our clinical studies, daily subcutaneous (SC) administration has decreased the relapse rate by 73.4 % from baseline. In the present study, relapsing MS patients were treated with 20 mg Copolymer 1 on alternate days. Sixty eight patients were recruited: 51 and 40 patients completed one and two years respectively. The relapse rate during the two years of treatment decreased by 80.8 % compared to the two years prior to treatment (means 0.56±1.02 vs 2.91±1.10, respectively, P<0.0001). The Expanded Disability Status Scale (EDSS) did not differ from baseline following the first year of treatment, but increased somewhat at the end of the second year (baseline=2.72±1.55, one year 2.71±1.59, two years 2.97±1.80, P<0.008). The drug was very well tolerated. This study suggests that alternate day therapy has beneficial effects and is well tolerated in relapsing Multiple Sclerosis, comparing favourably with the effects of daily injections of Copolymer 1.

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ALTERNATE DAY ADMINISTRATION OF COPOLYMER 1 FOR TREATING AUTOIMMUNE DISEASES

The present invention provides compositions and methods for treating an autoimmune disease which include administering a single dose of a therapeutically effective amount of Copolymer 1 (Cop 1) on alternate days.

RELATED APPLICATIONS

The present application claims the benefit of the filing date of provisional application 60/102,961 filed October 2, 1998 which is incorporated by reference herein.

BACKGROUND OF THE INVENTION

Autoimmune diseases occur when an organism's immune system fails to recognize some of the organism's own tissues as "self" and attacks them as "foreign." Normally, self-tolerance is developed early by developmental events within the immune system that prevent the organism's own T cells and B cells from reacting with the organism's own tissues. MHC cell surface proteins help regulate these early immune responses by binding to and presenting processed peptides to T cells.

This self-tolerance process breaks down when autoimmune diseases develop. Now the organism's own tissues and proteins are recognized as "autoantigens" and are attacked by the organism's immune system. For example, multiple sclerosis is believed to be an autoimmune disease occurring when the immune system attacks the myelin sheath, whose function is to insulate and protect nerves. It is a progressive disease characterized by demyelination, followed by neuronal and motor function loss. Rheumatoid arthritis ("RA") is also believed to be an autoimmune disease which involves chronic inflammation of the synovial joints and infiltration by activated T cells, macrophages and plasma cells, leading to a progressive destruction of the articular cartilage. It is the most severe form of joint disease. The nature of the autoantigen(s) attacked in rheumatoid

arthritis is poorly understood, although collagen type II is a candidate.

A tendency to develop multiple sclerosis and rheumatoid arthritis is inherited -- these diseases occur more frequently in individuals carrying one or more characteristic MHC class II alleles. For example, inherited susceptibility for rheumatoid arthritis is strongly associated with the MHC class II DRB I *0401, DRB 1 *0404, or DRB 1*0405 or the DRB1*0101 alleles. The histocompatibility locus antigens (HLA) are found on the surface of cells and help determine the individuality of tissues from different persons. Genes for histocompatibility locus antigens are located in the same region of chromosome 6 as the major histocompatibility complex (MHC). The MHC region expresses a number of distinctive classes of molecules in various cells of the body, the genes being, in order of sequence along the chromosome, the Class I, II and III MHC genes. The Class I genes consist of HLA genes, which are further subdivided into A, B and C subregions. The Class II genes are subdivided into the DR, DQ and DP subregions. The MHC-DR molecules are the best known; these occur on the surfaces of antigen presenting cells such as macrophages, dendritic cells of lymphoid tissue and epidermal cells. The Class III MHC products are expressed in various components of the complement system, as well as in some non-immune related cells.

A number of therapeutic agents have been developed to treat autoimmune diseases, including steroidal and non-steroidal anti-inflammatory drugs, for example, methotrexate; various interferons; and certain inhibitors of prostaglandin synthesis. However, these agents can be toxic when used for more than short periods of time or cause undesirable side effects. Other therapeutic agents bind to and/or inhibit the inflammatory activity of tumor necrosis factor (TNF), for example, anti-TNF specific antibodies or antibody fragments, or a soluble form of the TNF receptor. These agents target a protein on the surface of a T cell and generally prevent interaction with an antigen presenting cell (APC). However, therapeutic compositions containing natural folded proteins are often difficult to produce, formulate, store, and deliver. Moreover, the innate heterogeneity of the immune system can limit the effectiveness of drugs and complicate long-term treatment of hyper-immune diseases.

Thus in order to effectively treat autoimmune diseases and other immune conditions, new drugs are needed that do not have the side effects of the pres nt therapeutic agents and which adequately address to the innate heterogeneity of the immune system.

Multiple sclerosis may have a relapsing-remitting or a chronic progressive course. In the more common relapsing-remitting form the relapses may leave the patients with residual deficits, causing neurological disability that is accumulating over time. The aim of therapy in relapsing-remitting MS patients is to prevent exacerbation and the accumulation of disability. Recently, Cop 1, and interferon (IFN) ß-1a and IFN ß-1b, have been shown to decrease the relapse rate, and possibly slow down the disability of multiple sclerosis patients in double-blind, placebo controlled studies.

Cop 1 was the first drug to be introduced for the treatment of Multiple Sclerosis. Clinical double-blind, placebo controlled studies with Cop 1 showed beneficial effects when it was given by daily subcutaneous (SC) injections of 20 mg, reducing the relapse rate and slowing the progression of disability. The introduction into therapeutic studies, however, has bypassed the usual dose-finding phase and the dose and frequency of administration have been determined arbitrarily, from pilot studies which demonstrated efficacy and safety but provided little information on dosing intervals.

Accordingly, more precise dosing regimens are needed for Cop 1 to most effectively treat autoimmune diseases like multiple sclerosis.

SUMMARY OF THE INVENTION

The present invention provides a composition which includes a therapeutically effective amount of Copolymer 1 for treating an autoimmune disease which surprisingly can be administered on alternate day. This invention also provides a method of treating an autoimmune disease which comprises administering a single dose of a therapeutically effective amount of Cop 1 on alternate days. According to the present invention, the course of the autoimmune disease can be stayed or reversed by administering a therapeutically effective amount of Cop 1 on alternate days. In particular, the frequency of exacerbations

and the Expanded Disability Status Scale can be improved through use of the present compositions and methods.

REFERENCES

Aharoni, et al., 58 Immunology Letters 79 (1997).

Allison, in IMMUNOSUPPRESSION AND ANTI-INFLAMMATORY DRUGS, ANNALS OF THE NEW YORK ACADEMY OF SCIENCE 696:xi (1993).

Arnon R, et al., 25 Isr. J. Med. Sci. 686 (1989).

Ben-Nun A et al., 243 J NEUROL (Suppl 1) S14-S22 (1996).

Borenstein et al., 11 Ann Neurol 317 (1982).

Borenstein et al., 317 NEW ENGL. J. MED. 408 (1987).

Borenstein et al., 41 Neurology 533 (1991).

Clark, 21 J. CLIN PHARM & THERAP.195-199 (1996).

Dorling et al., 6 CUR. OPINIONS IMMUNOL. 765 (1994).

Ebers et al., 42 Ann of Neurology 986 (Abstract) (1997)(122nd Annual meeting of the American Neurological-Association, San Diego).

Ferrara et al., 324 NEW ENGLAND J. OF MEDICINE 667 (1991).

Fridkis-Hareli, et al., 63 J. NEUROCHEM. 63 (Suppl. 1) S61 (1994).

Fridkis-Hareli, et al., 163 CELL. IMMUNOL. 229. (1995).

Fridkis-Hareli, et al., 160 J. IMMUNOL. 4386 (1998).

Goodkin et al., MULTIPLE SCLEROSIS- ADVANCES IN CLINICAL TRIAL DESIGN, TREATMENT AND FUTURE PERSPECTIVES (Springer-Verlarg, Berlin 1996).

The IFN b multiple sclerosis study group, 43 NEUROLOGY 665 (1993).

Jacobs LD, et al., 39 ANN NEUROL 285-294 (1996).

Johnson et al., 45 NEUROLOGY 1268-1276 (1995).

Johnson, 1 NEUROLOGY 65-70 (1995).

Kay et al., 22 TRANSPLANTATION PROCEEDINGS 96 (1990).

Kelemen, et al., 102 INT ARCH ALLERGY IMMUNOL. 309 (1993).

Korczyn et al., 243 J NEUROL (Suppl 1): S 23-S 26.

Lublin, et al., 46 NEUROLOGY 12-18 (1996).

Meiner et al., *Copolymer I in relapsing-remitting multiple sclerosis: a multi-centre trial*, in O. Abramsky & H. Ovadia, eds., FRONTIERS IN MULTIPLE SCLEROSIS

- CLINICAL RESEARCH AND THERAPY 213-21 (Martin Dunitz 1997).

Mengle-Gaw, *The Major Histocompatibility Complex (MHC)*, in the ENCYCLOPEDIA OF MOLECULAR BIOLOGY 602-06 (Oxford: Blackwell Science Ltd., 1994).

Rothbard, J. B., et al,. 9 ANNU. REV. IMMUNOL. 527 (1991).

Rudik et al., 337 NEW ENG. J. MED. 1604 (1997).

Schlegel, et al., 84 BLOOD 2802 (1994).

Sela M et al., 88 BULL INST PASTEUR 303-14 (1990).

Stazl, 22 TRANSPLANTATION PROCEEDINGS 5 (1990).

Sykes, 10 THE FASEB JOURNAL 721 (1996) .

Teitelbaum et al., 1 EUR. J. IMMUNOL. 242-48 (1971).

Teitelbaum et al., 3 EUR. J. IMMUNOL. 273-79 (1973).

Teitelbaum et al., 64 J. NEUROIMMUNOL. 209-17 (1996).

Teitelbaum et al., 53 Cell. Mol. Life Scl. 24 (1997).

Thomson, 10 IMMUNOLOGY TODAY 6 (1988).

Van Den Bogaerde, et al., 52 TRANSPLANTATION 15 (1991).

Webb et al. 13 IMMUNOCHEM. 333 (1976).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the mean number of exacerbations observed in patients treated with Cop 1 during the two years prior to treatment and during the two years after treatment.

Figure 2 depicts the effect of the duration of Cop 1 treatment on the relapse rate.

Figure 3 depicts the mean difference of Expanded Disability Status Scale (EDSS) scores relative to the screening score.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a pharmaceutical composition for alternate day administration of Copolymer 1, comprising a single dose of a therapeutically effective amount of Cop 1 and a pharmaceutically acceptable carrier. The present invention further provides a method for treating autoimmune

diseases which includes administering a therapeutically effective amount of a polypeptide comprising amino acids tyrosine, glutamic acid, alanine and lysine.

Autoimmune diseases contemplated by the present invention include either cell-mediated disease (i.e. T-cell) or antibody-mediated disorders. Such disorders can be *inter alia* arthritic conditions, demyelinating diseases and inflammatory diseases. Autoimmune diseases which can be treated with the present compositions and methods include autoimmune hemolytic anemia, autoimmune oophoritis, autoimmune thyroiditis, autoimmune uveoretinitis, Crohn's disease, chronic immune thrombocytopenic purpura, colitis, contact sensitivity disease, diabetes mellitus, Graves disease, Guillain-Barre's syndrome, Hashimoto's disease, idiopathic myxedema, multiple sclerosis, myasthenia gravis, osteoarithritis, psoriasis, pemphigus vulgaris, rheumatoid arthritis, or systemic lupus erythematosus. The present compositions can be used to treat one or more of these diseases.

According to the present invention, the Cop 1 polypeptides generally consist of the amino acids tyrosine, glutamic acid, alanine or lysine. However, one of skill in the art can readily substitute structurally-related amino acids for these tyrosine, glutamic acid, alanine and lysine amino acids without deviating from the spirit of the invention. Thus, the present invention further contemplates conservative amino acid substitutions for tyrosine, glutamic acid, alanine and lysine in the present polypeptides. Such structurally-related amino acids include those amino acids which have about the same charge, hydrophobicity and size as tyrosine, glutamic acid, alanine or lysine. For example, lysine is structurally-related to arginine and histidine; glutamic acid is structurally-related to aspartic acid; tyrosine is structurally-related to serine, threonine and phenylalanine; and alanine is structurally-related to valine, leucine and isoleucine.

Polypeptides related to Cop 1 which are contemplated by the present invention include those polypeptides that are so structurally related to Cop 1 that they possess biological activity, e.g., the ability to suppress or eliminate B cell-mediated or T cell-dependent immune response, upon alternate day administration. As such, the term includes polypeptides which contain amino acids that are structurally-related to tyrosine, glutamic acid, alanine or lysine, so

long as the polypeptides still retain substantially equivalent biological activity in their ability to suppress or alleviate the symptoms of the autoimmune disease.

Moreover, the present polypeptides can be composed of *l*- or *d*-amino acids. As is known by one of skill in the art, *l*-amino acids occur in most natural proteins. However, *d*-amino acids are commercially available and can be substituted for some or all of the amino acids used to make the present polypeptides. The present invention contemplates Cop 1 formed from mixtures of *d*- and *l*- amino acids, as well as Cop 1 consistently essentially of either *l*- or *d*-amino acids.

According to the present invention, Cop 1 is preferably a polypeptide containing tyrosine, alanine, glutamic acid and lysine. The average molar fraction of the amino acids in Cop 1 can vary, for example, tyrosine can be present in a molar ratio of about 0.005 to about 0.250; alanine can be present in a molar ratio of about 0.3 to about 0.6; lysine can be present in a molar ratio of about 0.1 to about 0.5; and glutamic acid can be present in a molar ratio of about 0.005 to 0.300. The preferred average molar fraction of the amino acids is glutamic acid (0.141), alanine (0.427), tyrosine (0.095) and lysine (0.338), respectively. Basic Cop 1, rather than acidic Cop 1, is preferred. Cop 1 is preferably provided as an acetate salt of the polypeptide mixture.

The average molecular weight and the average molar fraction of the amino acids in the Cop 1 can vary, and may depend upon the method of administration. However, a molecular weight range of about 2,000 to about 100,000 is contemplated, with a preferred range of about 2,000 to about 40,000. More preferably, the molecular weight varies between about 3,000 to about 35,000 daltons. The most preferred average molecular weight is between about 5,000 and about 9,000 daltons. Preferred molecular weight ranges and processes of making the present polypeptides are also described in U.S. Patent No. 5,800,808, which is hereby incorporated by reference in its entirety.

The present Cop 1 polypeptides can be formulated into pharmaceutical compositions containing a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption

delaying agents, sweeteners and the like. The pharmaceutically acceptable carriers may be prepared from a wide range of materials including, but not limited to, flavoring agents, sweetening agents and miscellaneous materials such as buffers and absorbents that may be needed in order to prepare a particular therapeutic composition. The use of such media and agents with pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The present compositions may be formulated as an injectable, oral or spray solution or suspension.

EXAMPLE 1

METHODS

Patients

Patients were enrolled in four medical centres, after obtaining the approval of the ethical committees. All patients signed an informed consent form. Patients had clinically definite or laboratory-supported definite MS, with a relapsing-remitting or relapsing-progressive type and with at least two exacerbations during the two years prior to study entry. Patients had to be clinically stable for at least one month before entry. No steroids and other immuno-modulators could be used during six months prior to study entry. The Expanded Disability Status Scale (EDSS) score at baseline had to be less than 6.0. Both males and females aged 18 and over were eligible. Existence of any other chronic disease or pregnancy excluded patients from the study. Patients evaluation included physical and neurological examinations, laboratory evaluation (haematology, blood chemistry, urinalysis) and vital signs.

Drug supply

Cop 1 was manufactured by Teva® Pharmaceutical Industries Ltd. Israel. It was supplied as a sterile lyophilized material in single dose vials containing 22 mg of the active drug and 40 mg of mannitol. The medication was reconstituted before administration with diluent (sterile water). Patients or members of their family were instructed how to prepare and administer the medicine. Twenty milligrams of Cop 1 were administered every other day. New supplies of Cop 1 were provided at three months intervals during scheduled visits. On each scheduled and unscheduled visit adverse events were recorded. The neurological course of the disease was assessed by monitoring the annual relapse rate and the change of the EDSS score.

Premature discontinuation

Treatment was discontinued in any of the following circumstances: serious or intolerable adverse events; patient's decision to discontinue treatment for any reason; investigator's judgement that continuation of treatment is not in the best interest of the patient; poor compliance (less than 75%), disease progression (EDSS progression over 6.0) and loss to follow-up.

Statistics

The aim of the statistical analysis was to assess changes in efficacy parameters during the course of the treatment. Comparison of changes in EDSS score and in annual relapse rate were performed. Paired t-tests were conducted to examine whether the results differed significantly from zero.

RESULTS

Patient Characteristics

Table 1 demonstrates demographic and clinical characteristics of the 68 patients who were enrolled, consisting of 51 females (75%) and 17 males (25%). Mean age was 35.5 years (SD±9.8), ranging from 19 to 61 years. The mean age when MS symptoms first appeared was 28.5 years (SD±8.9) ranging from 14 to 52 years. The total number of self reported exacerbations prior to trial entry ranged from 2 to 20 (mean 5.8, SD±3,8). The mean number of exacerbations reported during the two years prior to trial entry was 2.9 (SD±1.5), ranging from 1 to 12. The mean baseline EDSS score was 2.7 (SD±1.5), ranging from 1 to 8.

Table 1. Demographic and Clinical characteristics of the patients treat d with Cop 1

	Cop 1 Patients
Gender: Male	17
Female	51
Age (yr) Mean ± SD	35.4±9.8
Range	19-61
Age when first symptom appeared (yr)	
Mean±SD	5.8±3.8
Range	2-20
Total number of relapses reported prior to trial	
Mean±SD	2.9±1.5
Range	1-12
EDSS score prior to onset of treatment	
Mean±SD	2.7±1.6
Range	1-8

Premature discontinuation

Table 2 summarizes the distribution of termination reasons. According to the open-label design of the study, discontinuation was allowed after one or two years. Of the 68 patients enrolled, 27 patients dropped out during the first two years of treatment: 8 (11.8%) due to adverse experience; 5 (7.3%) were lost to follow-up; 5 (7.3%) withdrew voluntarily; 6 (8.8%) due to investigator's judgement; 1 (1.5%) due to poor compliance, 1 (1.5%) due to disease progression and 1 (1.5%) due to normal conclusion.

Table 2. Terminati n reasons during the first two years of trial

Entered the trail	N (out of 68)	% of total
Adverse experience	8	11.8%
Termination due to investigator's judgement	7	10.3%
Voluntary withdrawal	6	8.8%
Lost to follow-up	5	7.3%
Poor compliance	1	1.5%
Still on trial at the end of the first year	53	77.9%
Still on trial at the end of the second year	41	60.3%

Relapses during the study

Table 3 displays mean values and ranges of the number of exacerbations per patient prior to the study and during the first two years of study (see also Figure 1). At the end of the first year, 64.7% of the patients who were still being treated remained relapse-free (33/51). At the end of the second year, 70.7% of the patients who continued throughout the second year (29/41) remained relapse-free. The mean relapse rate for the patients who completed two years of treatment (n=40) decreased from 2.9 (±1.1) in the two years prior to study entry to 0.56±1.02 at the end of second year. This represents 80.8% reduction in relapse rate, and is highly statistically significant (p=0.0001). The annual relapse rate during succeeding year decreased (See Figure 2).

Table 3a. Distribution of exac rbations at various lev ls

	Number of Patients	Total number of exacerbations	Mean ± SD	Range Min-Max
Two years prior to trial entry	68	195	2.9±1.1	1-12
During first two year of treatment	41	23	0.56±1.02	0-4

Table 3b. Distribution of patients free of relapses at various intervals

	Number of patients relapse free	%
End of first year of treatment	33/51	64.7%
End of second year of treatment	29/41	70.7%

Disability accumulation

Evaluation of the EDSS score during the study revealed that most of the patients who completed two years of treatment remained stable and only a few have deteriorated. Table 4 displays mean values, standard deviations and range of the EDSS scores at screening and following each year of treatment. Table 5 displays mean differences between EDSS scores at yearly intervals beginning at screening and ending after two years of treatment. The difference was computed by subtracting the value at an earlier interval from that obtained at the later interval. The results obtained showed that EDSS scores remained stable during the first year of treatment ($p=0.084$) and subsequently mildly deteriorated (see Figure 3).

Table 4. EDSS scores at yearly intervals

	N	Mean	SD	Min.	Max
Screening	68	2.72	1.55	1	8
After first year of treatment (last visit of first year)	53	2.71	1.59	1	7.5
After second year of treatment (last visit of second year	41	2.97	1.80	0	6.5

Table 5. Differences in EDSS scores at yearly intervals

	N	Mean difference	SD	P-value
Screening vs. First year of treatment	53	0.132	0.54	0.084
Screening vs. Second year of treatment	41	0.426	0.99	0.008
Screening vs. Third year of treatment	29	0.706	1.29	0.006
First year of treatment vs. Second year of treatment	41	0.29	0.78	0.021
First year of treatment vs. Third year of treatment	29	0.568	1.1	0.009
Second year of treatment vs. Third year of treatment	29	0.206	0.6	0.076

Adverse experience

Table 6 displays all adverse experiences recorded, coded according to the COSTAR system. More than 17% of all patients participating in the trial (12/68) did not report any adverse event during the study. Local injection site reactions were reported as 36.1% of the total number of reports (Table 6). An idiosyncratic, systemic adverse reaction, manifested by chest pain, palpitations and tachypnea was reported as 16.6% of the total number of adverse events reported. These sporadic, brief (2-20 minutes) reactions occurred immediately following drug administration and resolved without any treatment. Additional

systemic reactions, such as rash (1.8%) and lymphadenopathy (1.8%) were rarely reported. Most of the adverse experiences reported were considered to be mild (51.5%) or moderate (29.5%) in nature. Only 9.9% (33/332) were considered severe: vasodilation 5/332 (1.5%), dizziness 4/332 (1.2%), palpitations 3 /332 (0.,9%), dyspnea 2/332 (0.6%), pruritus 2/332 (0.6%), rash 2/332 (0.6%), headache 2/332 (0.6%), injection site edema 2/332 (0.6%), accidental injury 1/332 (0.3%), chills 1/332 (0.3%), injection site mass 1/332 (0.3%), deep thrombophlebitis 1/332 (0.3%), myocardial infarction 1/332 (0.3%), pulmonary embolus 1/332 (0-3%), dysphagia 1/332 (0.3%), vomiting 1/332 (0.3%), manic reaction 1/332 (0.3%), amblyopia 1/332 (0.3%) and breast carcinoma 1/332 (0.3%). The majority of adverse experiences reported (67.2%) were considered by the investigators to be probably related to treatment with Cop 1; 7.2% were considered to be possibly related, while the rest were considered to be unrelated or of unknown cause. Adverse experiences were reported most commonly during the first six months of treatment. The majority of the adverse experiences (76.8%) were reported as resolved at the time of completion of the study, in 8.4% with sequel. No clinically significant changes were observed in any of the routine laboratory examinations.

Table 6: Incidence and frequency of adverse experiences

	Number of Reports	% of reports	Number of patients	% of patients
Total	332	100%	56	82.4%
Injection site reactions*	120	36.1%	34	60.7%
Idiosyncratic systemic adverse reaction	38	11.4%	16	28.6
Rash	6	1.8%	4	7.1%
Lymphadenopathy	6	1.8%	1	1.8%

*Including local sensitivity (10.5%), pain (7.8%), edema (6.0%), mass (4.8%), atrophy (3.6%), inflammation (0.6%), hemorrhage (0.6%), cyst (0.3%), and other local site reactions (1.8%).

The study reported here was methodologically identical to the previous open label study performed by us, except that injections were administered in alternate days rather than daily. The results were surprisingly similar to those of the previous study, in terms of both efficacy and tolerability.

Thus, treatment with Cop1 on alternate days is as effective as daily treatment and can have even fewer side effects and be more benign than is treatment with IFN ß. In the interferon study, 12MIU were not significantly better than 6MIU, indicating that the 6MIU IFN ß dosage is the maximum effective dosage – and that interferon has a ceiling effect. The present studies indicate that 20 mg of Cop 1 on alternate days has a "ceiling effect." Alternatively it is possible that the biological effect of Cop 1 is not dose related but is time related to the exposure of the immune system to its presence and the continuity of administering the drug with rechallenging the immune system, thus making daily injection unnecessary.

The results of this study support previous reports that Cop 1 is a safe drug. Most of the adverse events reported were mild and transient. Adverse experiences were reported by 56 (82.4%) of the patients, the most frequent being mild local injection site reactions, which were reported by 34 patients (50%). Sixteen patients reported 38 episodes of one or more symptoms of transient self-

limited reactions (i.e., palpitations, flushing, dyspnoea or chest pain) which resolved spontaneously within a short time. These symptoms were also reported in placebo-controlled Cop 1 studies, and did not follow any recognizable pattern of appearance, recurrence and disappearance. Placebo patients reported similar reactions but less frequently. Such adverse reactions are likely due, at least in part, to anxiety reactions. Eight patients (11.8%) withdrew from the study because of adverse experiences. Seven of them were felt to be possibly or probably related to treatment with Cop 1. There were no deaths during the study. The frequency of adverse experiences reported decreased after the first 6 months of therapy.

CONCLUSIONS

The results of this trial indicate that:

1. Treatment with 20 mg Cop 1 in alternate day injections subcutaneously is safe and well tolerated.

2. Relapse rate decreases significantly and stabilizes at lower levels.

3. Patients' disability, as measured by EDSS, does not deteriorate during the first year of treatment, although deterioration was observed during the second year. It may very well be that the natural history of the disease is attenuated by Cop 1.

4. The results obtained in this open label-study, with alternate day injection of Cop 1, showed safety and efficacy as well, as the daily injection studies reported in several controlled trials.

What is Claimed:

1. A method of treating an autoimmune disease which comprises administering a single dose of a therapeutically effective amount of Copolymer 1 on alternate days.

2. The method of Claim 1 wherein said Copolymer 1 comprises a polypeptide consisting essentially of amino acids tyrosine, alanine, lysine and glutamic acid.

3. The method of Claim 1 wherein said tyrosine is present in a molar ratio of about 0.005 to about 0.250; said alanine is present in a molar ratio of about 0.3 to about 0.6; lysine is present in a molar ratio of about 0.1 to about 0.5; and said glutamic acid is present in a molar ratio of about 0.005 to about 0.300.

4. The method of Claim 1 wherein said Copolymer 1 has a molecular weight of about 2,000 to about 40,000 daltons.

5. The method of Claim 1 wherein said Copolymer 1 has a molecular weight of about 5,000 to about 9,000 daltons.

6. The method of Claim 1, wherein said autoimmune disease is an arthritic condition, a demyelinating disease or an inflammatory disease.

7. The method of Claim 1, wherein said autoimmune disease is autoimmune hemolytic anemia, autoimmune oophoritis, autoimmune thyroiditis, autoimmune uveoretinitis, Crohn's disease, chronic immune thrombocytopenic purpura, colitis, contact sensitivity disease, diabetes mellitus, Graves disease, Guillain-Barre's syndrome, Hashimoto's disease, idiopathic myxedema, multiple sclerosis, myasthenia gravis, osteoarithritis, psoriasis, pemphigus vulgaris, rheumatoid arthritis, or systemic lupus erythematosus.

8. The method of Claim 1 wherein said therapeutically effective amount of Copolymer 1 is 1.0 mg to 40.0 mg.

9. The method of Claim 1 wherein said therapeutically effective amount of Copolymer 1 is 10.0 mg to 30.0 mg.

10. The method of Claim 1 wherein said therapeutically effective amount of Copolymer 1 is about 20 mg.

11. A composition for treating an autoimmune disease by alternate day administration which comprises a therapeutically effective amount of Copolymer 1 and a pharmaceutically acceptable carrier formulated for administration on alternate days.

12. The composition of Claim 11 wherein said Copolymer 1 comprises a polypeptide consisting essentially of amino acids tyrosine, alanine, lysine and glutamic acid.

13. The composition of Claim 11 wherein said tyrosine is present in a molar ratio of about 0.005 to about 0.250; said alanine is present in a molar ratio of about 0.3 to about 0.6; lysine is present in a molar ratio of about 0.1 to about 0.5; and said glutamic acid is present in a molar ratio of about 0.005 to about 0.300.

14. The composition of Claim 11 wherein said Copolymer 1 has a molecular weight of about 2,000 to about 40,000 daltons.

15. The composition of Claim 11 wherein said Copolymer 1 has a molecular weight of about 5,000 to about 9,000 daltons.

16. The composition of Claim 11, wherein said autoimmune disease is an arthritic condition, a demyelinating disease or an inflammatory disease.

17. The compostion of Claim 11, wherein said autoimmune disease is autoimmune hemolytic anemia, autoimmune oophoritis, autoimmune thyroiditis, autoimmune uveoretinitis, Crohn's disease, chronic immune thrombocytopenic purpura, colitis, contact sensitivity disease, diabetes mellitus, Graves disease, Guillain-Barre's syndrome, Hashimoto's disease, idiopathic myxedema, multiple sclerosis, myasthenia gravis, osteoarithritis, psoriasis, pemphigus vulgaris, rheumatoid arthritis, or systemic lupus erythematosus.

18. The composition of Claim 11 wherein said therapeutically effective amount of Copolymer 1 is 1.0 mg to 40.0 mg.

19. The composition of Claim 11 wherein said therapeutically effective amount of Copolymer 1 is 10.0 mg to 30.0 mg.

20. The composition of Claim 11 wherein said therapeutically effective amount of Copolymer 1 is about 20 mg.

Figure 1

MEAN NUMBER OF EXACERBATION FOR TWO YEARS PRIOR TO STUDY AND TWO YEARS SUBSEQUENT TO TREATMENT

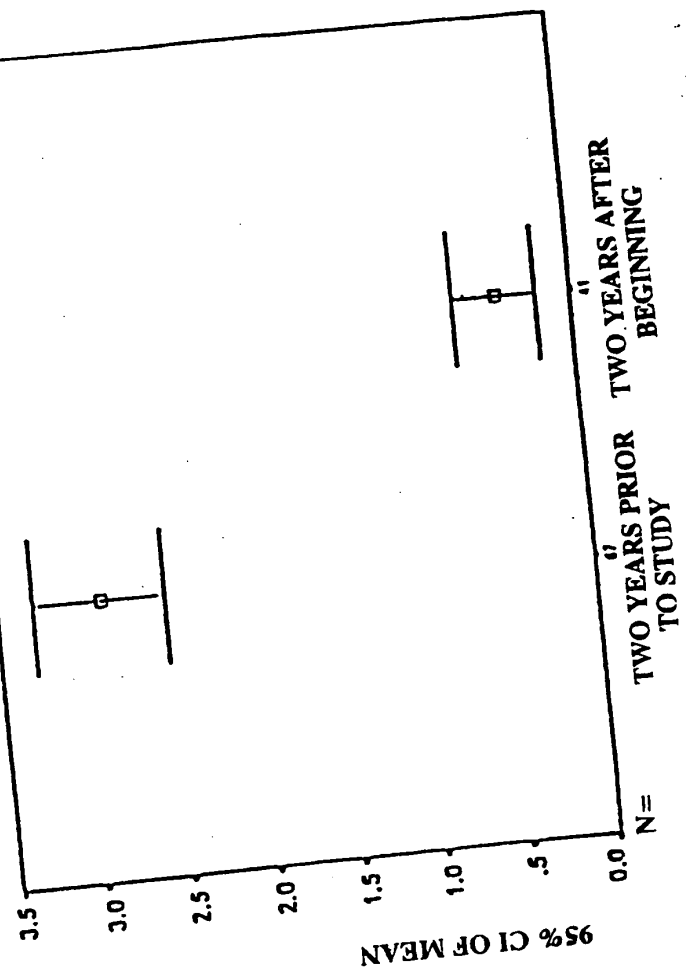


Figure 2

EFFECT OF TREATMENT DURATION ON RELAPSE RATE

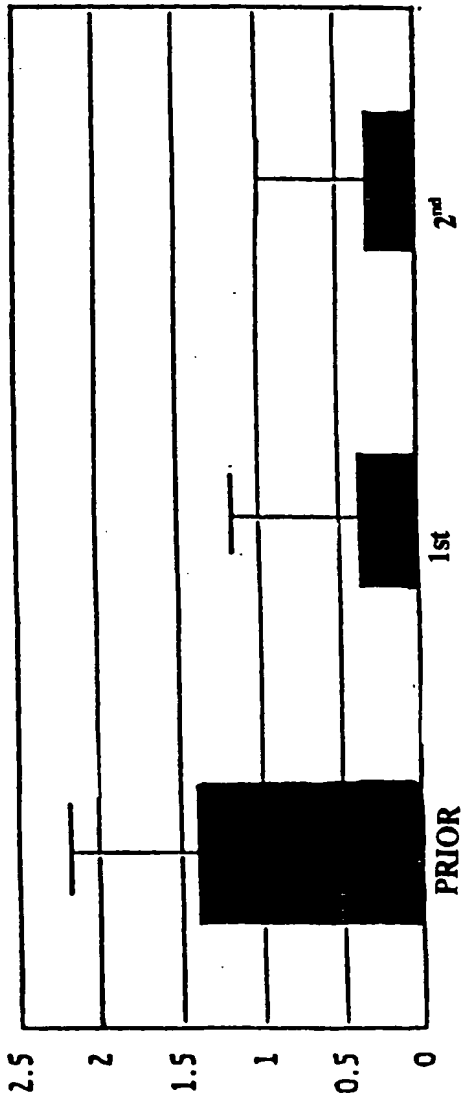
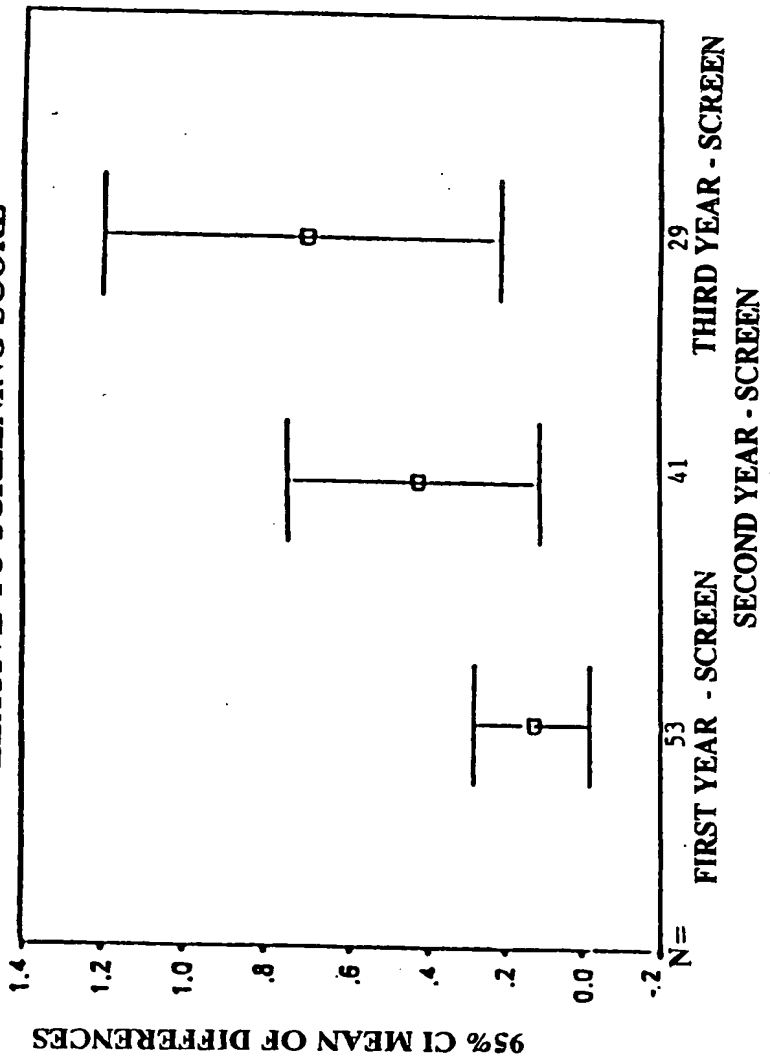


Figure 3

MEAN DIFFERENCES OF EDSS SCORES
RELATIVE TO SCREENING SCORE



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/22836

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/74
US CL :424/78.08
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/78.08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,800,808 A (KONFINO et al.) 01 September 1998, see column 1, lines 11-22 and 64-68 and column 2, lines 1-7 and 42-48.	1-10
X		11-20
A	US 5,668,117 A (SHAPIRO) 16 September 1997.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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